almost every field examined (Figs. 1 and 2). The stained organisms stood out very clearly against the counterstained background on the smear. It was apparent that the organisms were pleomorphic in this preparation, since both rather long rods and coccobacillary forms were observed.

Confirmation of the presence of *L. monocytogenes* in this specimen was made by culture. The isolated strain satisfied the criteria for *L. monocytogenes* and was designated type 1 by M. L. Gray, Montana State College, Bozeman. It stained very readily with this conjugated polyvalent antiserum (Fig. 3).

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SULFATE-FREE GROWTH OF *CLOSTRIDIUM NIGRIFICANS*

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Certain strains of the mesophilic, nonsporulating sulfate-reducing bacteria (*Desulfovibrio desulfuricans*) grow without detectable sulfate if pyruvate is the main substrate [Postgate, Research (London) **5**:189, 1952] but the sporulating mesophile *D. orientis* does not (Adams and Postgate, J. Gen. Microbiol. **20**:252, 1959). This note reports evidence that the sporulating thermophilic sulfate-reducing bacterium *Clostridium nigrificans* (Campbell, Frank, and Hall, J. Bacteriol. **73**:516, 1957) is capable of sulfate-free growth with pyruvate.

Strains were tested in the basal medium of Postgate (1952), without the 2.5% NaCl there prescribed and with 54 mM sodium lactate or pyruvate (reagent grade) and 27 mM Na_2SO_4. Nine strains were examined: Teddington Garden (NCIB 8351), Delft 74T [NCIB 8395; incorrectly named "14T" by Campbell et al. (1957)], both originally isolated as "*Sporovibrio thermodesulfuricans*"; ATCC strain 3750, ATCC strain 7946, both originally isolated as food-spoilage *C. nigrificans*; strains 55, 106, 134, "By," and "Dp" from the personal collection of L. L. Campbell. All strains were pure according to the criteria of Postgate (J. Gen. Microbiol. **9**:440, 1953). The strains were maintained under N_2 at 55 C in Baars's lactate-sulfate medium (Postgate, J. Sci. Food Agr. **10**:669, 1959) containing 0.1% yeast extract; supernatant fluids from 24- to 48-hr cultures were used as inocula for growth tests. Growth was assessed turbidimetrically in a Klett-Summerson photoelectric colorimeter at 660 mμ.

All nine strains grew in pyruvate media with or without sulfate in less than 18 hr; on prolonged incubation, some decline in optical density occurred with most strains. Growth with lactate was sometimes delayed up to 24 hr and never appeared earlier than with pyruvate; growth did not occur with lactate but no sulfate. Examples of growth yields are recorded in Table 1; strain 55 regularly gave low cell yields with lactate but gave "normal" yields with pyruvate. Data demonstrating the dependence of growth of strain Teddington Garden on pyruvate concentration in the sulfate-free medium are also included in Table 1; this experiment was performed employing the specially purified sodium pyruvate mentioned below. Six strains (those listed in Table 1 with "By" in place of 55) were subcultured six times in sulfate-free pyruvate medium without change in the cell yield; on the sixth passage, they were also returned to Baars's medium, in which they grew and reduced sulfate readily.

For the fourth subculture, the sodium pyruvate was recrystallized from 80% ethanol and the cultures were analyzed after growth. Acetate, the sole volatile acid produced, was characterized by paper chromatography in *n*-butanol + 1 N

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NH_4OH (1:1, v/v) and estimated after steam distillation in a Markham apparatus; pyruvate was estimated as the 2,4-dinitrophenylhydrazone (Grossman and Postgate, J. Gen. Microbiol. **12**:429, 1955). Table 2 records that five strains converted pyruvate stoichiometrically to acetate. Strain Teddington Garden was grown 40 hr in vacuo and, after acidification to 0.2 N with H_2SO_4, the gas was collected under water; gas-phase chromatography by N. Ryckman showed that it was H_2 plus CO_2. Ratios of H_2 to CO_2 formed are given in Table 2 and were determined in the following manner. On their sixth passage in sulfate-free pyruvate medium, the substrate concentration was lowered to 36 mM and the five strains were grown in 10-ml batches in duplicate evacuated Thunberg tubes, one with 0.5 ml of 2 N H_2SO_4 in the side arm, the other with 0.25 ml of 10 N NaOH. After growth at 55 C for 44 hr, the contents of the side arms were tipped, the tubes shaken well, and stood 30 min to allow complete absorption or evolution of CO_2. They were then opened under water, and the remaining gas volumes were measured at atmospheric pressure. These experiments show that *C. nigrificans* is capable of sulfate-free growth at the expense of the classical anaerobic fission of pyruvate to hydrogen, CO_2, and acetate.

TABLE 1. *Growth of Clostridium nigrificans with and without sulfate**

Strain	Lactate + Na_2SO_4	Pyruvate + Na_2SO_4	Pyruvate
Teddington Garden†	78	72	33
Dp	73	32	59
3750	67	33	32
134	75	56	30
106	35	81	70
55	8	89	53

* Supernatant fluid from 24-hr cultures of the indicated strains in Baars's lactate-sulfate medium were inoculated into media based on the substrates below and incubated at 55 C under N_2 for 66 hr. Carbon sources: 54 mM; Na_2SO_4: 27 mM. Growth was recorded in Klett units at 660 mμ corrected for "blank" growth (5 to 9 units).

† Strain Teddington Garden after its fourth passage in sulfate-free pyruvate medium was subcultured into media prepared with different concentrations of purified sodium pyruvate and incubated at 55 C under N_2 for 48 hr. Results were as follows: with no pyruvate, growth was 6 Klett units; with 4.5 mM pyruvate, 10 Klett units; with 9 mM, 15 units; with 18 mM, 28 units; with 36 mM, 55 units; with 54 mM, 66 units.

TABLE 2. *Acetate, CO_2, and hydrogen yields from fermentation of pyruvate by Clostridium nigrificans**

Strain	Acetate	Pyruvate	CO_2-H_2 (v/v)
	mM	mM	
Teddington Garden	52	0.3	1.04
3750	54.5	1.4	0.89
134	47	0.025	1.14
106	50	0.7	1.08
Dp	48	1.0	0.85

* On their fourth passage in sulfate-free pyruvate medium, the strains indicated were grown with 54 mM purified sodium pyruvate; the cultures were analyzed for acetate and residual pyruvate after 66 hr at 55 C. On their sixth passage, they were grown in evacuated vessels and the H-CO_2 ratios were determined volumetrically (see text).

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OCCURRENCE OF UNUSUAL SALMONELLAE IN LABORATORY MICE

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During the examination of fecal samples from laboratory mice for the identification and elimination of salmonella carriers, we encountered serotypes which have not been previously reported as occurring in this animal species.

Isolations were made from fecal samples by a